ANTIBACTERIAL ACTIVITY OF SOME NATURAL DUHOKIAN HONEY AGAINST CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS, IRAQ

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ABSTRACT

Antibiotic resistance of bacteria is on the rise, namely multi-resistant Staphylococcus aureus which are resistant to all of the antibiotics in common use. Thus the discovery of alternative therapeutic agents is urgently needed. Honey is an ancient wound treatment that was re-introduced into modern medical practice and possesses therapeutic potential, including wound healing properties and antimicrobial activity. The aim of this study is to investigate and comparison of antibacterial activity of four types of natural honey, found in Duhok city, with commercial one against a multi-resistant Staphylococcus aureus strains isolated from wounds and burns infections in Duhok city, Iraq. Methods A total of 100 wound and burn swabs were collected and analyzed by screened on Blood, Mannitol salt, MacConkey and Nutrient agar followed by the identification of the isolates based on their cultural characteristics and their reactions in standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique according to the Clinical and Laboratory Standards Institute guidelines on Muller Hinton Agar. The antibacterial activity of four natural honeys (agriculture college honey, Barwari Bala, Emadia and Shingal) and one commercial Al-Sheefa honey was evaluated by agar diffusion assay and determination of MIC values. Of the 100 swabs, 25 (25%) swab yielded positive culture of Staphylococcus aureus; the organism exhibited high resistance rates to all used antibiotics. The all natural honeys exhibited highest antibacterial activity than commercial one. Moreover, agriculture college honey had excellent antibacterial activity, at very low concentration 5% (wt/vt) produce inhibition zones, followed by Barwari Bala, Emadia then Shingal honey. Minimal inhibitory concentration (MIC) values of four types of natural honeys were found to be 5, 25, 50 and 50 % (v/v) for AC honey, Barwari Bala, Emadi and Shingal honey respectively. This study highlights that some natural Duhokian honey has complete and partial antibacterial
activity when tested in vitro against a multi-resistant Staphylococcus aureus strains isolated from wounds and burns infections.

Key words: Multi-resistant Staphylococcus aureus, wounds and burns infections, honey

INTRODUCTION

Multi-resistant Staphylococcus aureus are frequently isolated from skin wounds and involved in difficult-to-treat skin and underlying tissue infections namely methicillin-resistant Staphylococcus aureus (MRSA). Anti-infective drugs are critically important in reducing the global burden of infectious diseases. Antibiotic resistance of bacteria is on the rise, namely multi-resistant S aureus which are resistant to all of the antibiotics in common use. However, as resistant microbes develop and spread, the effectiveness of the drugs is diminished\(^1\). This type of resistance to antimicrobial agent is an increasing problem in many areas of the world especially in developing countries\(^2\) - \(^3\). Therefore, it is necessary to protect patients with impaired immunity from exposure to them. Thus, the discovery of alternative therapeutic agents is urgently needed.

The use of traditional medicine to treat infection has been practiced since the origin of mankind, and in past it was the only method available\(^4\). Honey produced by honeybees (Apis mellifera) is one of the oldest traditional medicines considered to be important in the treatment of respiratory ailment, gastrointestinal infection and various other diseases. It is being used effectively as a dressing for wounds, (including surgical wounds), burns, and skin ulcers to reduce pain and odor quickly. Honey is an ancient wound treatment that was re-introduced into modern medical practice and possesses therapeutic potential and antimicrobial activity\(^5\). To date, no extensive studies of the antibacterial properties of honey, found in Duhok city, Iraq, against multi-resistant S aureus isolated from wounds and burns infection have been conducted. Therefore, the purpose of the present study was designed to evaluate the in vitro antimicrobial potential of some types of natural honey with different sources in Duhok city, Iraq, against a multi-resistant S aureus strains isolated from wounds and burns infections. Also, the comparison of antibacterial activity of natural honeys with commercial honey was studied.

MATERIALS AND METHODS

Study Population:

A total of 100 samples were obtained from those outpatients who showed an evidence of surgical wound and burn infections in Azadi teaching hospital and Burn hospital in Duhok city; Iraq, between May 2012 to August 2012.

Sample Collection:

Pus samples / wound swabs were collected with aseptic precautions from clinically suspected postoperative wound infections and burn infections then transported to the laboratory without delay. The specimens have been routinely processed by the laboratory
of Microbiology section at faculty of medical sciences in Duhok University, Duhok city, Iraq.

Bacterial Identification:

For this purpose, several media and tests were used for the isolation, identification and testing the susceptibility of the isolates for common used antibiotics. The media used are: blood (with 5-7% defibrinized blood), chocolate, MacConkey, DNase, nutrient, mannitol salt and Mueller-Hinton agar. Coagulase, catalase, tests were used for the identification. All of the above media and reagents were obtained from (Difco. USA). The media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 20 min. All specimens (wound swabs and pus) were inoculated onto sheep blood, chocolate, mannitol salt agar and MacConkey agar plates and incubated aerobically at 37°C for 18-24 hours. The incubation was extended to at least 48-72 hours for discernible colony development. Identification of the isolates was done using standard procedures as follow:

Morphological characteristics and gram stain were performed. To check the growth pattern blood agar (with 5-7% defibrinized blood), MacConkey agar, mannitol salt agar were used. For biochemical characteristics, DNA hydrolysis, sugar fermentation, coagulase, catalase, oxidase test and novobiocin disc (5 g) were performed.

Antibiotic susceptibility tests:

Antibiotic susceptibility tests were carried out on isolated and identified colonies of S. aureus isolates using commercially prepared antibiotic sensitivity disc (Oxoid, England) using modified Kirby-Bauer method according to CLSI guidelines, using Mueller-Hinton agar standard media. The inhibition zone standards for antimicrobial susceptibility were considered from tables for interpretative zone diameters of Clinical and Laboratory Standards Institute (CLSI). Antibiotics used were: amoxicillin (10 g), amoxiclav (10 g), ciprofloxacin (100 g), erythromycin (5 g), co-trimaxazole (25 g), nitrofurantoin (10 g), doxycycline (10 g), vancomycin (10 g), chloramphenicol (10 g), oxacillin (30 g) and cephalixin (30 g).

Honey:

The antibacterial properties of four types of natural Duhokian honey (Agriculture College honey( ACH), Barwari Bala honey, Shingal honey and Emadia honey) against different clinical isolates of S. aureus were determined by comparison to the commercially available AL-Sheefa honey (Saudi Arabia made). Natural Duhokian honey; agricultural college honey was get from honeybees of agricultural college at Duhok university, Emadia honey from emadia district, Shingal honey from shingal district and both Barwari Bala honey and commercial one Al-Sheefa were obtained from Mustapha Market at Zerkha neighborhood, Duhok city, Iraq. A sterile mesh was used to remove debris and then streaked on blood agar plate, and incubated overnight to check microbial purity and stored at 2-8 °C until used.
Preparation of Honey Solutions:

Honey solutions were prepared immediately prior to testing by diluting honey to the required concentrations (100, 50, 25, 12.5 and 5% wt/vl).

Antimicrobial activity of honey samples:

The agar well diffusion method was employed. Solidified nutrient agar plates were separately flooded with the liquid inoculums of the different test organisms using the spread plate method. The plates were drained and allowed to dry at 37°C for 30 min after which four equidistant wells of 5 mm in diameter were punched using sterile cork borer at different sites on the plates. A 50 micro liter of the different concentrations (100, 50, 25, 12.5 and 5% wt/vl) of the honey samples were separately placed in the punched wells with 1 ml sterile syringe. The plates were allowed to stay for 15 min for pre-diffusion to take place followed by an overnight incubation that lasted for 24 h at 37°C and growth compared to a control plate that contained no sample (National Committee for Clinical Laboratory Standards, 1999). The diameter of inhibition zones, including the diameter of the well, was recorded. Each assay was carried out in triplicate. All strains were handled under aseptic conditions and the microorganisms were destroyed by autoclave to ensure bio-safety.

Minimal Inhibitory concentration Determination (MIC):

Concentrations of honey suspension (100, 50, 25, 12.5 and 5% vol/vol) were incorporated into media to test their efficiency against different clinical isolates of S. aureus. Each plate reaching final volume 5 ml including both media and honey was inoculated and incubated at 37°C for 48h. The MIC was determined by finding the plate with lowest concentration of honey on which the strain would not grow. All MIC value were expressed in % (v/v).

RESULTS

Of the 100 surgical wound and burn swabs cultured, 25 (25%) yielded positive culture of Staphylococcus aureus. All specimens were directly transferred to the microbiology laboratory and cultured to the appropriate media (as described in methods).

Table 1 shows the susceptibility rates of Staphylococcus aureus against eleven used antibiotics. A majority of clinical isolates showed high susceptibility rates to chloramphenicol, nitrofurantoin and vancomycin with moderate susceptibility to oxacillin and doxycyclin. On other hand, most other used antibiotics were ineffective.

In this study, we compared the antibacterial activity of four natural Dohukian honey (ACH, Barwari Bala, Emadia and Shingal) with commercial Al-Sheefa honey (Sudia Arabia made) by determination of inhibition zone and MIC values.
Table 1. Antibiotic Susceptibility Test of *Staphylococcus aureus* isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Symbol</th>
<th>Disc potency (µg)</th>
<th>Susceptibility rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>C</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>F</td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>VA</td>
<td>10</td>
<td>66</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>OX</td>
<td>30</td>
<td>53</td>
</tr>
<tr>
<td>Doxycyline</td>
<td>DO</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>Co-trimoxoal</td>
<td>SXT</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>E</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>CL</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>AMV</td>
<td>10</td>
<td>0.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AM</td>
<td>10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 2 shows the antibacterial activity, using agar diffusion assay, of various concentrations of four natural honeys (ACH, Barwari Bala, Emadia and Shingal) and one commercial Al-Sheefa honey against clinical isolates of *S. aureus* from wounds and burns infection. The all natural honey exhibited highest antibacterial activity than commercial one. Moreover, among natural honeys there was a marked variation in the level of antibacterial activity. AC honey had excellent antibacterial activity followed by Barwari Bala, Emadia then Shingal honey. Agricultural college (AC) honey showed zones of inhibition of 15mm at very low concentration 5% (wt/vt). While variable results were obtained with remainder types of honey and no inhibition zone at various concentrations was obtained with Al-Sheefa honey.

Table 2. Antimicrobial activity of “Barwari Bala”, “Shingal”, “AC honey”, “Emadia” and “AL-Sheefa” honey on 25 clinical isolates of *Staphylococcus aureus*.

<table>
<thead>
<tr>
<th>Type of honey</th>
<th>Honey Concentrations Diameter of inhibition zone in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% (wt/vl)</td>
</tr>
<tr>
<td>AC honey</td>
<td>30* (15**)</td>
</tr>
<tr>
<td>Barwari Bala</td>
<td>30 (20)</td>
</tr>
<tr>
<td>Emadia</td>
<td>20(17)</td>
</tr>
<tr>
<td>Shingal</td>
<td>30(2)</td>
</tr>
<tr>
<td>AL-Sheefa</td>
<td>NZ</td>
</tr>
</tbody>
</table>

* Inhibition zone in mm
**number of isolates given inhibition zone out of 25 isolates
NZ; no inhibition zone

Table 3 shows the MIC values of four natural honeys and one commercial honey against clinical isolates of *S. aureus* from wounds and burns infection. MIC values of four types of natural honeys were found to be 5, 25, 50 and 50 % (v/v) for AC honey, Barwari Bala,
Emadi and Shingal honey respectively. AC honey had lower MICs (indicating better activity) than other honey types against 25 of the tested bacteria.

Table 3. Minimum Inhibitory Concentration (MIC) of different honeys against clinical isolates of *Staphylococcus aureus*.

<table>
<thead>
<tr>
<th>Type of honey</th>
<th><em>Staphylococcus aureus</em> MIC% (vol/vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%(vol/vol)</td>
</tr>
<tr>
<td>AC honey</td>
<td>15*</td>
</tr>
<tr>
<td>Barwari Bala</td>
<td>13</td>
</tr>
<tr>
<td>Emadia</td>
<td>13</td>
</tr>
<tr>
<td>Shingal</td>
<td>10</td>
</tr>
<tr>
<td>AL-Sheefa</td>
<td>0,0</td>
</tr>
</tbody>
</table>

* Number of inhibited isolates out of 25 isolates

**DISCUSSION**

It has been documented that honey has a bacteriostatic and bactericidal effect against various species of both gram positive and gram negative bacteria, as well as an antifungal effect. Using a pathogen like *Staphylococcus aureus*, with innate or acquired antibiotic resistance, tested to their sensitivity to honey is a critical issue due to resistance to honey could not be induced under conditions that rapidly induced resistance to antibiotics. In this study, the isolation rate of culture positive of *S. aureus* from wound and burn samples was 25% and most of them were multiple resistant, i.e. resistant to more than half numbers of used antibiotics. Antimicrobial activity of four natural types of Duhokian honeys and commercial one in present survey was evaluated by measuring the zone of inhibition (mm) against *S. aureus* which showed significant variations. Inhibition zone (30 mm) of *S. aureus* occurred at honey concentrations 100% and 50% (w/v) with all four types of natural honey. Moreover, marked growth inhibition and greatest antibacterial activity has been recorded with AC honey even at very low concentration 5%. This insight highlights if this honey used as wound contact layer and diluted 20-fold by wound exudates it still ceases bacterial growth and to be inhibitory. Moreover, numerous interesting observations on the clinical use of honey have been successfully eradicated methicillin-resistant *S. aureus* from colonized chronic wounds. On another hand, the lowest concentrations to produce inhibition zone for Barwari Bala, Emadia and Shingal honey were 25%, 50% and 50%(w/v) respectively in our survey. A study in India surveyed antibacterial activity on *S. aureus* of five type of natural honey (Baidhyanath honey, Uttarakhand honey, Dabur, Wings honey and Alwar) at different concentration 20%, 40%, 60%, 80% and 100%, found that Baidhyanath followed by Dabur honey were more effective even at low concentration 20% exerted antimicrobial activity and produced inhibition zones 20 and10 mm respectively. This is already accord with. An another work by Nzeako and Hamdi (2000) on six commercial honeys found that zone inhibition of *S. aureus* did not occur at honey concentrations 40% but occurred at above concentrations. In other data, zone inhibition of *S. aureus* was found.
Antibacterial activity of honey against *Staphylococcus aureus* at concentration up to 30% of natural honey. Similar research conducted in Malaysia recorded inhibition zone (12.0 to 13.0 mm) at the honey concentration of 5.48 mg ml and 2.74 mg ml respectively, against *S. aureus* in disc diffusion method. Again, the our experiment showed that Al-sheefa honey (commercial honey) in high concentration did not exerted antibacterial activity as natural honey, indicate lacking of factors that are operating to provide the observed antibacterial effect or might be supersaturated sugar or artificial honey. This result similar to they concluded that the antibacterial activity of honey was greater than that which could be attributed to the sugar content of the honey.

The variation in the antimicrobial potential of honey samples used in present study as compared to the previous similar studies highlights that the source of the nectars may have contributed to the difference in the antimicrobial activities of honey that is, the flowers from which bees gathered nectar to produce the honey, since flora source determines many of the attributes of honey, for example flavor, aroma, color and composition of honey might be highly variable.

In this mini-survey, minimal inhibitory concentration (MIC) values of four types of natural honeys against isolates of *S. aureus* were found to be 5, 25, 50 and 50% (v/v) for AC honey, Barwari Bala, Emadi and Shingal honey respectively. AC honey had lower MICs (indicating better activity) than other honey types against 25 of the tested bacteria. Molan demonstrated MIC value of Manuka honey needed to completely prevent growth of *S. aureus* was 1.8% (V/V). Other work found MIC value against *S. aureus* was 6.5%. MIC of Malaysian honey, Tualang was (20%) against *S. aureus*. These results indicating that there is a variation in the antimicrobial potency of honey. Over and above, the results shown by honey samples in relation to *S. aureus* may be important, notably in recent decades a marked increase in difficult to treat skin and underlying tissue infections with *S. aureus* due to high developed resistance against several common antibiotics. Thus, new strategies to treat wounds infected with *S. aureus* are needed, and the possibility to use honey appears as a convenient and less costly treatment option.

**CONCLUSION**

In conclusions, some natural Duhokian honey that used here has complete and partial antibacterial activity when tested *in vitro*. However, pharmacological standardization and clinical evaluation on the effect of honey are essential before using honey as a preventive and curative measure to common diseases related to the tested bacterial species. The wider availability of honey in the rural our areas provide its utilization for certain diseases.

**COMPETING INTERESTS**

The authors declare no competing interest.

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